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Drought and Salinity Impacts on Bread Wheat in a Hydroponic Culture: A Physiological Comparison

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Abstract

Drought and salinity are two major abiotic stresses, similarly and/or differently affecting physiological processes of wheat. The aim of this study was to evaluate and compare the impacts of drought and salinity on wheat. A pot experiment was conducted as completely randomized design with three replications in the research greenhouse of Yasouj University in 2015. Treatments included different levels of salinity and drought with the same osmotic potentials (-2.47, -4.94 and -7.42 bar) and a control. Salinity and drought were imposed with NaCl and PEG 6000 in a Hogland medium, respectively. Results showed that by increasing drought and salinity treatments, relative water cotent and cell membrane stability were decreased but malondialdehyde (MDA) increased. The effect of PEG drought stress on these traits was more than that of NaCl stress. Increasing drought and salinity stresses significantly increased leaf proline, total soluble sugars, and glycinebetaine content, however, this increase was higher for salinity. Fv/Fm was equally affected by salinity and drought, decreasing by both stresses. By raising stress levels, chlorophyll a decreased but chlorophyll b and carotenoid content increased. In general, we found that wheat could tolerate acceptable salinity levels better than drought, by accumulation of osmolytes and more sustained absorption of water and also reducing the MDA production under salinity conditions.

Keywords: Chlorophyll; Glycinebetaine; Malondialdehyde; Proline; Soluble sugars

Introduction

Wheat is one of the most strategic and major food crops all over the world, and different stresses reduce its yield (Curtis and Halford 2014). Stress is a result of disturbances in physiological processes that can be achieved from one or a combination of biotic and abiotic factors. Excessive soil salinity causes ionic and osmotic stresses, leading to physiological damage to plants. Plants grown under salinity will be under ionic stress, which is the result of the accumulation of sodium in the leaves (Yu *et al*. 2015). Salinity can reduce wheat growth and yield by adverse effects on ion distribution, photosynthesis and the availability of water for plant (Pervize *et al*. 2002; Okcu *et al*. 2005). Salinity decreases the relative water content (RWC) in wheat, but salt tolerate cultivars can keep RWC better in stress conditions by osmotic regulation (Qasim *et al*. 2003).

The plasma membrane is the first site that suffers under stress conditions (Levitt *et al*. 1980). As a result of damage to cell membranes, leakage of materials increases and ultimately the stability of cell membranes reduces and so cell death occurs (Blume and Ebercon 1981). Malondialdehyde (MDA) production due to destruction of cell membranes is a response of plants to environmental stresses, especially salinity (Munns 2002). MDA is the final product of cell unsaturated lipid peroxidation, so it is used as a useful biomarker to determine the lipid

peroxidation and oxidative stress in cells. Salinity may affect the function of the photosynthetic mechanism, particularly photosystem II (Li *et al*. 2010).

Drought stress is one of the abiotic stresses affecting different stages of plant growth. Drought is one of the major constraints to agricultural productivity worldwide. Photosynthesis, the most basic physiological process in green plants, is also strongly influenced by this stress at all stages. Mesophyll cells dehydration occurs in severe drought stress conditions and causes a strong inhibition of photosynthetic processes. Drought stress also reduces mesophyll cell efficiency for using the available carbon dioxide (Ashraf and Harris 2013). Water stress at different stages after pollination increases lipid peroxidation and decreases both the stability of the membrane and the amount of chlorophyll and carotenoids (Sairam and Saxena 2000). Drought stress causes a significant decrease in photosynthetic rate and leaf pigment content, including chlorophyll and carotenoids (Colom and Vazzana 2003). Proline is an amino acid that plays an important role in regulating osmotic adjustment and accumulates in the leaves faster than the roots under stressful conditions. Proline accumulation may result from further degradation of proteins and sensitivity of cell to drought stress. Proline accumulation under drought stress in plants such as peas (Ayerbe and Tenorio 1998; Ghorbanali et al. 2001) and canola (Ferreira and Lourens 2002) has been reported. Soluble sugars are also compatible osmolytes that accumulate in stress conditions and protect the cells by osmotic adjustment and the stability of the membranes. In fact, plants under stress use various ways to deal with the stress. Accumulation of other compatible osmolytes such as glycinebetaine is the other way for stress tolerance (Rhoads and McIntosh 1991). In general, secondary metabolites, including the carotenoids, improve the plant defense mechanism against stresses, especially oxidative stress induced by the high salt content (Lim *et al*. 2012). Ashraf and Harris (2013) mentioned that carotenoids (Car) are necessary for photoprotection of photosynthesis and they play an important role as a precursor in signaling during the plant development under abiotic/biotic stress. They have a significant potential to enhance nutritional quality and plant yield. The aim of this study was to compare the impacts of different levels of salinity and drought, with the same osmotic potential, on some physiological characteristics of winter wheat.

Materials and Methods

The experiment was conducted as a completely randomized design with three replications in the research greenhouse of Yasouj University in 2015. Treatments included different levels of salinity and drought with the same osmotic potentials (-2.47, - 4.94 and -7.42 bar) each at three levels along with a control treatment. Salinity and drought were imposed by using sodium chloride and polyethylene glycol 6000, respectively.

The amount of NaCl needed for providing osmotic potentials of solution was determined by van't Hoff equation (Taiz and Zeiger 1991); in addition, the amount of PEG needed for making concomitent drought solutions was obtained by the following equation (Michel and Kaufman 1973):

$$\Psi = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, CT + (8.39 \times 10^{-7})\, C^2T$$

Where, Ψ is PEG osmotic potential (bar), C is gram PEG per kg water and T is temperature as ^{O}C.

The wheat cultivar Falat was used in this experiment. Falat is a spring type and early maturing cultivar adapted to arid and semi-arid regions of Iran (Rezvani Moghaddam *et al.* 2015). The seeds were disinfected with 1% sodium hypochlorite and then were washed with distilled water, and finally 10 seeds were planted at a depth of 1.5 cm in each plastic pot, having been filled with fine and washed sand. The pots were irrigated with water and ¼ Hoagland solution with pH= 7 before and after germination up to 3-leaf stage, respectively (Hoagland and Arnon 1950). Hoagland nutrient solution was first used as ¼ Hoagland and then became ½ Hoagland until the end of the experiment. Salinity and drought stresses were imposed by adding to the pots the necessary amounts of NaCl for salinity levels and polyethylene glycol (PEG 6000) for drought levels from the 4-leaf stage until the end of the experiment (8-leaf stage). To prevent the accumulation of salts and nutrients in the soil, the pots were irrigated by 200 ml of distilled water once a week until the end of the experiment. Sampling was done on the fully expanded, youngest leaves at the end of the experiment, and some physiological characteristics were measured.

Determination of membrane lipid peroxidation was performed by measuring MDA via the method of Heath and Packer (1968). RWC was calculated by the following equation (Weatherley 1950):

$$\%\ \mathrm{RWC} = \frac{(\text{Fresh weight} - \text{Oven dried weight})}{(\text{Turgor weight} - \text{Oven dried weight})} \times 100$$

Cell membrane stability (CMS) was measured as Blum and Ebercon (1981, using 10 pieces of the sample leaves with a 3-cm diameter and leaf proline content was determined through Paquine and Lechasseur (1979) method, using 0.5 gr of fresh leaf. To measure the soluble sugars, the method by Irigoyen *et al.* (1992) was applied, utilizing 0.5 gr of fresh leaf. In order to measure the amount of glycinebetaine, Grattan and Grieve's method (1992) was employed, using 0.5 g of dry leaf tissue. Chlorophyll fluorescence measurements were performed on the fully expanded youngest leaves by a fluorimeter device (OS1-FL). The chlorophyll and carotenoids were measured as of Arnon (1949) and Lichtenthaler (1987), respectively.

Statistical analysis of the experimental data was done by SAS version 9.1.3 software, and mean comparisons were carried out *via* the least significant difference (LSD) test at $p \leq 0.05$.

Results

Results indicated that the effect of experimental treatments was significant for all traits except Fv/Fm (Table 1).

Table 1. Mean squares obtained from variance analysis of physiological traits in wheat leaves under different levels of salinity and drought

SOV	df	RWC	Cell membrane stability (%)	MDA	Proline	Soluble sugars	Glycinebetaine
Treatment	6	1026.0**	1174.3**	397.5**	7.3**	1342.8**	5.3**
Error	14	17.9	8.8	0.63	0.18	13.3	0.20
CV%	-	6.5	7.3	4.3	5.2	7.0	14.7

**Significant at p≤ 0.01; RWC: Relative water content; MDA: Malondialdehyde

Table 1 Continued

SOV	df	Fv/Fm	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
Treatment	6	0.007 ^{ns}	0.658**	0.032**	0.566**	0.0023**
Error	14	0.002	0.027	0.0006	0.096	0.0002
CV%	-	7.58	14.6	12.48	19.7	5.56

ns and **Non-significant and significant at p≤ 0.01, respectively

Leaf relative water content

The results showed that by reducing osmotic potential, the RWC decreased in both salinity and drought stress treatments compared to the non-treated (control) pots; however, the reduction in the drought treatment levels was much higher than the salinity levels (Figure 1a). In relation to salinity levels, the maximum and the minimum RWC were obtained from -2.47 and -7.42 bars, respectively; and there was no significant difference between -2.47 and -4.94 bars. The different drought treatments showed a significant falling trend for RWC and were in the range of 70.16% for the osmotic potential of -2.47 bar down to 34.08% for the osmotic potential of -7.42 bar (Figure 1a). The group comparisons between total salinity and total drought treatments and different levels of salinity and drought with the same osmotic potential showed significant differences in terms of RWC.

Cell membrane stability

The results showed that CMS decreased by increasing the levels of salinity and drought in comparison to the control treatment. The effect of salinity on CMS was significant, and by reducing osmotic potential from -2.47 to -7.42 bar, cell membrane stability decreased approximately 35% (Figure 1b). Furthermore, drought significantly reduced CMS so that statistically significant differences between various levels of drought were observed. The difference between the highest CMS (45.43%) in the drought treatment of -2.47 bar and the lowest (12.54%) in the drought treatment of -7.42 bar were almost 72% (Figure 1b). Based on Table 2, group differences between the salinity and drought treatments and also different levels of salinity and drought with the same osmotic potential were significant regarding CMS. The lowest CMS was obtained from salinity and drought group treatments with the same osmotic potential of -7.42 bar. It was also found that drought stress imposed higher damages on the membranes, which may be due to lower

cell RWC during drought stress (Figure 1a). In fact, following the increasing production of reactive oxygen species and changing the integrity of membranes by lipid and protein peroxidation, CMS drops significantly.

Table 2. Means squares obtained from variance analysis of group comparisons between treatment levels for wheat leaf physiological characteristics

Group comparisons	df	RWC	Cell membrane stability (%)	MDA	Proline	Soluble sugars	Glycinebetaine
Salinity(S) with drought(D)	1	926**	186.5**	122.2**	13.36**	2338.5**	6.34**
-2.47(S) with -2.47(D)	1	12641**	708.7**	326.6**	2.76**	2238.4**	4.28**
-4.94(S) with -4.94(D)	1	1231**	803.4**	58.4**	6.63**	2324.2**	4.33**
-7.42(S) with -7.42(D)	1	322**	980.4**	43.1**	4.38**	138.3**	0.045*

*,**Significant at p≤ 0.05 and p≤ 0.01, respectively; RWC: Relative water content; MDA: Malondialdehyde

Table 2 Continued

Group comparisons	df	Fv/Fm	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoid
Salinity with drought	1	0.0042^{ns}	0.364**	0.0046*	0.261^{ns}	0.0000^{ns}
-2.47(S) with -2.47(D)	1	0.0022*	0.0192^{ns}	0.0104^{ns}	0.0037^{ns}	0.0000^{ns}
-4.94(S) with -4.94(D)	1	0.0001^{ns}	0.0322^{ns}	0.0000**	0.0170^{ns}	0.0002^{ns}
-7.42(S) with -7.42(D)	1	0.0062^{ns}	1.1792**	0.0001^{ns}	0.912^{ns}	0.0002^{ns}

ns, *, **Non- significant and significant at p ≤ 0.05 and p≤ 0.01, respectively.

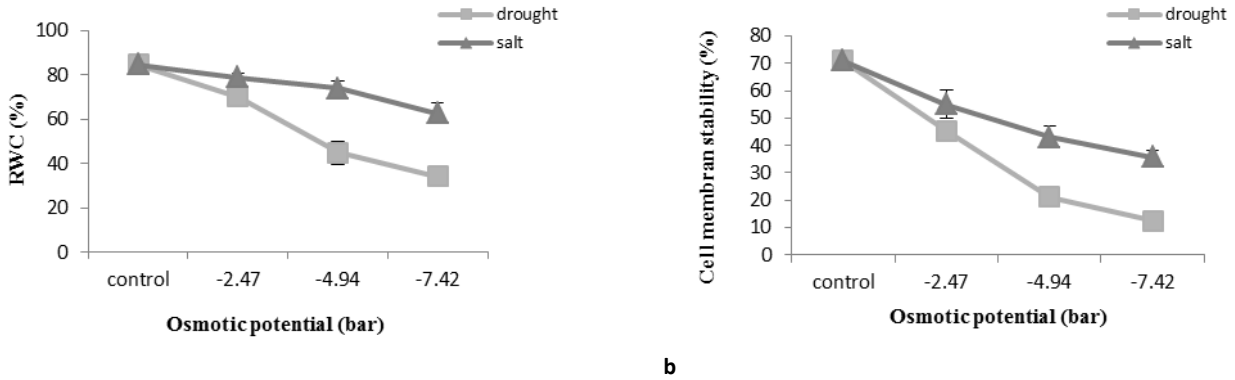

Figure 1. The effect of drought and salinity osmotic potentials on leaf relative water content (RWC)(a) and cell membrane stability (b) of wheat leaf. Vertical bars represent standard error ±SE

Malondialdehyde

The results revealed that decreasing osmotic potential by salinity and drought, significantly heightened MDA. At all of the osmotic potential levels, the impact of drought was higher than salinity (Figure 2a), which is related to smaller RWC in the drought condition. Group comparisons between the total salinity and drought treatment as well as salinity and drought with the same osmotic potentials showed

significant differences for MDA, and it was observed that the highest MDA belonged to salinity and drought with the same osmotic potential of -7.42 bar; additionally, the negative impact of drought stress was more than that of salinity. It is clear that owing to the greater impact of drought on the integrity and stability of cell membranes (Figure 2a), MDA production rises due to lipid peroxidation reactions.

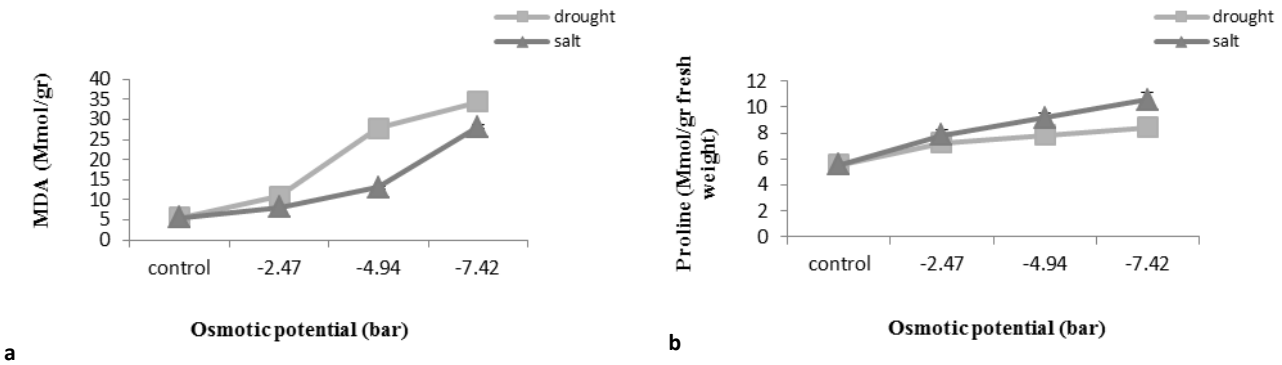

Figure 2. The effect of drought and salinity osmotic potentials on Malondialdehyde Production (a) and proline (b) of wheat leaf. Vertical bars represent standard error ±SE

Leaf proline content

The results showed that salinity and drought stresses increased leaf proline content as compared to the control. The maximum and the minimum proline contents were obtained from -7.42 and -2.47 bars, respectively; and the differences between maximum and minimum values were almost 25.6% (Figure 2b). In terms of drought stress, the maximum and the minimum proline contents were obtained from -7.42 and -2.47 bars, respectively, and there was no significant difference between -2.47 and -4.94 bars (Figure 2b). It was observed that group comparisons between the total salinity and total drought treatments as well as salinity and drought with the same osmotic potentials revealed significant differences for the proline content. The maximum amount of proline was obtained from salinity and drought with the same osmotic potential of -7.42 bars; besides, the effect of salinity on this trait was higher than that of drought (Figure 2b).

Leaf glycinebetaine content (LGBC)

The control treatment had the lowest LGBC, and decreasing the osmotic potential for both drought and salinity treatments, enhanced LGBC (Figure 3a). The results indicated that among different levels of salinity stress, the maximum and the minimum LGBC were for -7.42 and -2.47 bars, respectively. The effect of drought stress on LGBC proved that by decreasing the osmotic potential from -2.47 to -7.42 bars, LGBC increased almost 52%. Based on Table 2, group differences of glycinebetaine between total salinity and total drought treatments and also different levels of salinity and drought with the same osmotic potential were significant, and the largest LGBC was obtained from salinity and drought group treatments with the same osmotic potential of -7.42 bar; moreover, salinity stress

increased LGBC more than the drought stress (Figure 3a). As in the case of proline and soluble sugars, glycinebetaine is concentrated in the saline conditions more than the drought ones.

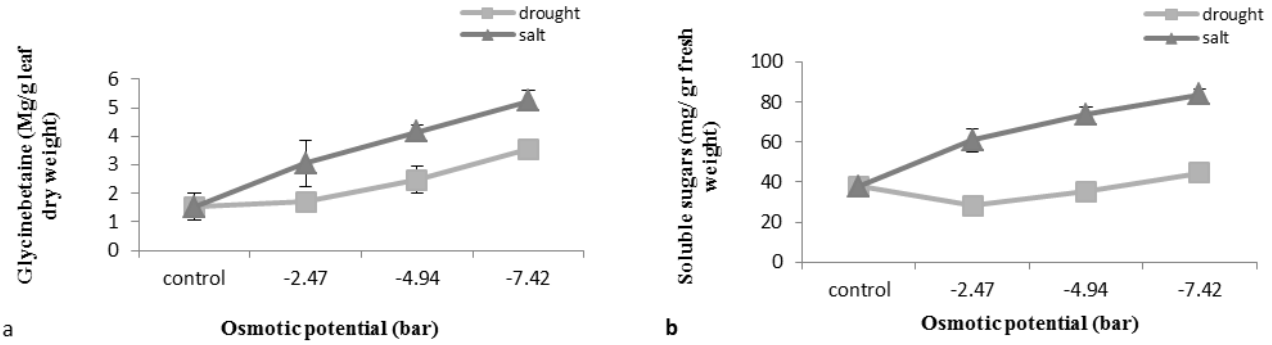

Figure 3. The effect of drought and salinity osmotic potential on glycinebetaine (a) and soluble sugar (b) of wheat leaf. Vertical bars represent standard error±SE

Leaf soluble sugars Content (LSSC)

Mean comparison showed that there were significant differences between control treatment and salinity and drought treatments for LSSC. Among salinity treatments the maximum and the minimum amounts belonged to -7.42 and -2.47 bars, respectively (Figure 3b). In general, LSSC significantly increased by heightening the salinity levels. At the salinity stress, by decreasing the osmotic potential up to -7.42 bars, LSSC showed an increasing trend. The maximum and the minimum amounts belonged to -7.42 and -2.47 bar, respectively. The difference between the minimum and the maximum LSSC was 15.33% (Figure 3b). Based on Table 2, group comparisons of soluble sugars between total salinity and total drought treatments and also different levels of salinity and drought with the same osmotic potential showed significant differences, and the highest LSSC was obtained from salinity and drought group treatment with the same osmotic potential of -7.42 bar. It was observed that salinity stress increased soluble sugars more than the drought stress (Figure 3b).

Leaf chlorophyll content

Chlorophyll a: Reduction of osmotic potential significantly decreased chlorophyll a. The highest amount of chlorophyll a was obtained in the control treatment, and for salinity the difference between -2.47 and -7.42 bars was significant. For drought, by reducing the osmotic potential from -2.47 to -7.42 bars, the amount of chlorophyll a significantly reduced about 94.67% (Figure 4a). The results of the group comparisons (Table 2) revealed that the difference between total salinity and total drought groups well as salinity and drought group treatments with the same osmotic potential of -7.42 bar were significant for chlorophyll a. The lowest amount of chlorophyll a was obtained from salinity and drought group treatments with the same osmotic potential of -7.42 bar. It was observed that salinity and drought had the same decreasing trend for chlorophyll a (Figure 4a).

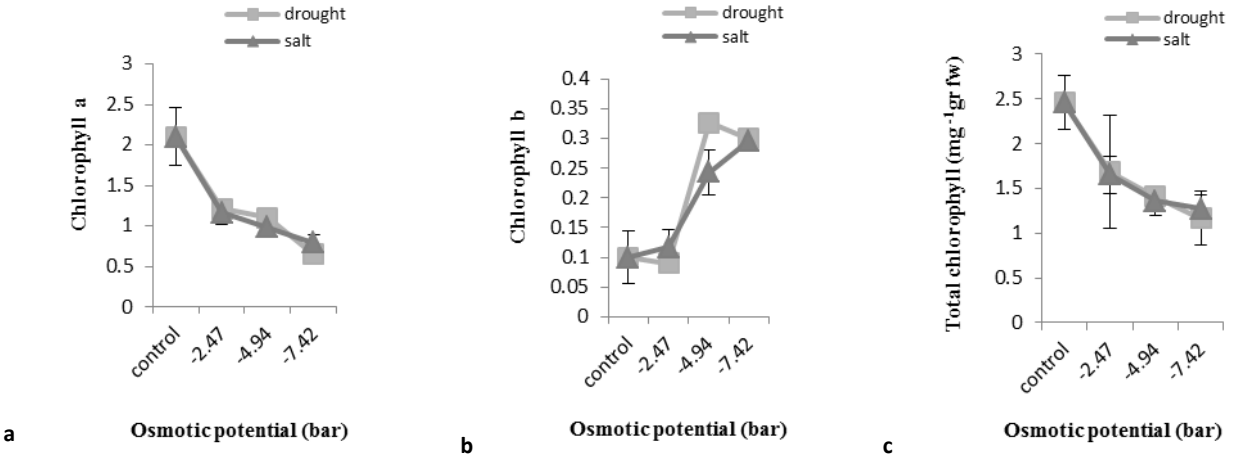

Figure 4. The effect of drought and salinity osmotic potential on chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) of wheat leaf. Vertical bars represent standard error ±SE

Chlorophyll b: Results presented the rise of chlorophyll b by decreasing osmotic potential after -2.47 bar for both salinity and drought treatments. In terms of salinity, maximum chlorophyll b was for the treatment with osmotic potential of -7.42 bars, and for drought, the highest chlorophyll b was obtained from the treatment with osmotic potential of -4.94 bar (Figure 4b). The change in the chlorophyll b was in contrast to the chlorophyll a. Mean comparisons made between group treatments (Table 2) showed that the difference of total salinity and total drought group treatments and also salinity and drought group treatments with the same osmotic potential of -4.94 bars were significant in terms of the amount of chlorophyll b, and the negative impact of drought stress was higher for this trait.

Total Chlorophyll: The results of total chlorophyll were the same as those of chlorophyll a and confirmed that chlorophyll b comprises a small proportion of total chlorophyll (Figure 4c). However, it can also be due to the redirection of nitrogen synthesis pathway to the formation of osmotic regulator compounds such as proline (Kaya *et al.* 2001). The results of the group comparisons (Table 2) showed that none of the treatment group differences was significant for this trait, and that salinity and drought were equally effective in reducing the amount of total chlorophyll.

Carotenoids

The results proved that the carotenoids increased by decreasing osmotic potentials for both drought and salinity similarly (Figure 5). Group comparisons (Table 2) showed that none of group treatment differences were significant for carotenoids, and that both salinity and drought had the same effect on carotenoids.

Discussion

As mentioned before, by reducing osmotic potential, RWC was reduced in both salinity and drought stress conditions, compared to the non-treated control. The main reason for RWC reduction with increasing levels of salinity, can be the decrease in water availability due to osmotic potential reduction caused by the accumulation of salts in the root rhizosphere. The reduction in RWC due to water stress is related to the production of abscisic acid hormone in roots and its accumulation in stomatal guard cells and stomata closure under water stress. Schonfeld *et al*. (1988) stated that drought stress reduced leaf water potential and RWC of leaf due to reduced absorption of water from the roots in the plants. It was also observed that the negative effect of drought stress on this trait was more than that of salinity stress. Indeed, at salinity levels, plants retain water absorption by reducing osmotic potential of the cells *via* salt absorption.

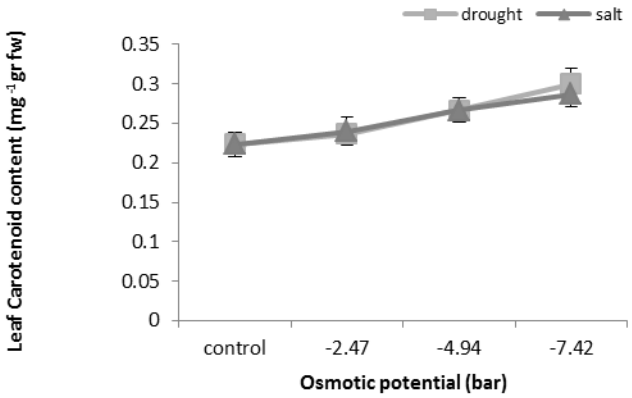

Figure 5. The effect of drought and salinity osmotic potentials on carotenoids of wheat leaf. Vertical bars represent standard error ±SE

CMS decreased by increasing the levels of salinity and drought in comparison with the control treatment. It seems that salinity and drought stresses caused the oxidative damage through the production of free oxygen radicals in cells, and these free radicals attacked proteins, lipids and nucleic acids and also decreased CMS of the cell membrane, causing the cytoplasmic leakage. Masoumi *et al*. (2010) reported that the drought stress led to damage the integrity of the cells and cell membranes *via* disrupting the function of reactive oxygen species scavenging systems. We found that the impact of drought on CMS was higher than salinity. We can also see that the bigger the RWC, the more the CMS was (Figure 1 a,b).

Decreasing osmotic potential by salinity and drought, significantly heightened MDA. Munns and James (2003) confirmed that in drought conditions, cell membrane integrity was damaged and MDA increased by production of reactive oxygen species and membrane lipid peroxidation. Jiang and Hung (2001) reported that under drought and heat stresses, MDA concentration increases due to the rise in lipid peroxidation and oxidation of membrane fatty acids. The inverse

relation between CMS and MDA could be seen from Figure 2 a. The more MDA content can illustrate the reduction in CMS.

Salinity and drought stresses increased leaf proline content compared to the control. Sannada *et al*. (1995) indicated that in barley, wheat and *Mesembryanthemum crystallinum* L. in the salinity condition, an increase in the proline concentration was because of synthesis of proline or a decrease in oxidation of proline to glutamate or degradation of protein to proline. Increasing the levels of proline under salt stress conditions is also because proline is a compatible osmolyte that scavenge free oxygen molecules generated during environmental stresses and protect macromolecules (Rahdari *et al*. 2012). Proline increasing under stress conditions may be due to further degradation of proteins and maybe sensitivity to drought stress. Kao (1981) showed that under stress, the protein of mature leaves degrades and their concentration reduces, leading to an increase in proline. In fact, with regard to the role of proline in overcoming the adverse effects of environmental stresses, especially salinity and osmotic potential (NasirKhan *et al*. 2007), this increase was expected. More proline in the salt condition compared to the drought condition shows that the protective role of proline in salinity condition is probably more than the drought condition.

The control treatment had the lowest LGBC, and decreasing the osmotic potential for both drought and salinity treatments, enhanced LGBC. As mentioned previously, plants use several different ways to overcome stresses under severe environmental conditions. Production and accumulation of compatible osmolytes such as glycinebetaine is one of these ways (Rhoads and McIntosh 1991). Khan *et al.* (2000) surveyed the effect of salinity on atriplex and confirmed that by increasing salt concentration, LGBC increased. Glycinebetaine accumulates in plants under stress conditions and acts as an osmotic adjustment in the plant, and its concentration increases with increasing salinity and drought (Hanson *et al*. 2007). Schobert (1977) stated that the attachment of glycinebetaine to hydrophobic domains of proteins and the water layer that forms around the proteins can be available at the time of stress and may prevent protein degradation.

In general, LSSC significantly increased by heightening the salinity levels. Soluble sugars are members of the compatible osmolytes that will increase by stresses and protect the cells through osmotic adjustment and stability of membranes. An increase in the concentration of soluble sugars plays a role in salt tolerance under salinity stress (Geholt *et al*. 2005). Eshghizadeh *et al*. (2014) in reviewing the effect of salinity on some physiological characteristics of millet, pointed out the effect of salinity on the increase in concentration of soluble sugars. Prado *et al.* (2000) considered the increase in the amount of soluble carbohydrates as a rout to reduce the adverse effects of osmotic and ionic conditions, and finally adaptation of plants to the stresses. It seems that the accumulation of these solutes is important in the maintenance of mechanisms such as restoration and compensation of lost volume of cells and the reduction of cell damages caused by free radicals as well as protection and stability of enzymes and membrane structure. The upsurge in

LSSC was also observed in soybean (Fututoku and Yamada 1981) and sorghum (Newton *et al*. 1986) due to the drought stress. The increase in LSSC in response to water stress can be attributed to lower transferring of LSSC from leaves and slower consumption because of growth reduction and other changes such as starch hydrolysis (Kameli and Losel 1996). The surge in LSSC is a mechanism that causes osmotic potential to decrease more in the cytoplasm, and helps the Na^+ to be removed from vacuoles and also causes osmotic adjustment (Orcutt and Nilsen 2000). More accumulation of proline, glycinebetaine and LSSC in the salt stress condition finely describes the reason for higher CMS and RWC and lower MDA content in saline than drought conditions.

Reduction of osmotic potential significantly decreased chlorophyll a. There are some reports on intensified activity of chlorophyllase enzymes that degrade chlorophyll under drought stress. One of the main reasons for the drop in chlorophyll a is their degradation by reactive oxygen species. These free radicals cause oxidation (Wise and Naylor 1989), resulting in degradation and decomposition of these pigments (Schutz and Fangmeir 2001). Our results showed the rise in chlorophyll b by decreasing osmotic potential for both salinity and drought treatments. The change in chlorophyll b was in contrast to chlorophyll a. It was observed that total chlorophyll was the same as those of chlorophyll a and confirmed that chlorophyll b consisted a small proportion of total chlorophyll. Zhao *et al*. (2007) demonstrated that salinity reduced the total amount of chlorophyll in oat, because salinity can reduce the synthesis or increase the degradation of chlorophyll in the leaves. In another study, it was revealed that reducing the chlorophyll concentration in spinach cultivars under salinity occurs because of common synthesis pathways of chlorophyll and alpha-tocopherol. The plant stopping chlorophyll biosynthesis under salinity stress activates the biosynthesis of alpha-tocopherol antioxidant instead. Anjum *et al*. (2003) showed that the total chlorophyll increased by drought stress in barley. They also stated that the drought reduces chlorophyll b but increases the stability of chlorophyll a, which in turn will increase the total amount of chlorophyll. In many salt tolerant species total chlorophyll content is increased and Ashraf and Harris (2013) suggested that this increase under salt stress could be a biochemical indicator for the salt tolerance in some plant species.

Carotenoids increased by decreasing osmotic potentials for both drought and salinity similarly. Carotenoids are responsible for cleaning oxygen free radicals, and their high levels in the cells can indicate the relative tolerance to stresses. Lim *et al*. (2012) illustrated that carotenoid levels in black wheat increased seven days after sowing in response to NaCl, and carotenoid levels in treatment groups of 50 and 100 mM NaCl were doubled in comparison with the control treatment. It is evident that carotenoids act as a factor for photoprotection by helping to the dispersion of excess energy.

Conclusion

The results showed that by increasing the levels of salinity and drought treatments, RWC and cell membrane stability decreased, however, the effect

of drought stress on the traits under study was more detrimental than the salinity stress. The highest MDA was achieved from salinity and drought treatments with osmotic potential of -7.42 bar. Furthermore, the negative effect of drought stress on MDA was more than the salinity stress. Leaf proline, soluble sugars and glycinebetaine content under salt and drought stresses were more than those of the control treatment, and the impact of salinity on these traits was higher. The minimum Fv/Fm was acquired from salinity and drought with osmotic potential of -7.42 bar. The impact of both drought and salinity treatments on this trait was similar. Reduction of osmotic potential significantly decreased chlorophyll a and total chlorophyll but increased chlorophyll b. However, for chlorophyll a and total chlorophyll, there were not significant differences between drought and salinity treatments at each osmotic potential. Leaf carotenoids content also exhibited an increasing trend by increasing drought and salinity levels, and both drought and salinity treatments had the same effects on leaf carotenoid content.

References

Anjum F, Yaseen M, Rasul E, Wahid A and Anjum S. 2003. Water stress in barley (*Hordeum vulgare* L.). II. Effect on chemical composition and chlorophyll contents. Pakistan Journal of Agricultural Science 40: 45-49.

Arnon DI, 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24 (1): 1-15.

Ashraf M and Harris PJC, 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51 (2): 163-190.

Blum A and Ebercon A, 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21: 43-47.

Colom MR and Vazzana C, 2003. Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environmental and Experimental Botany 49 (2): 135-144.

Curtis T and Halford NG, 2014. Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. Annals of Applied Biology 164 (3): 354–372.

Eshghizadeh HR, Kafi M, Nezami A and Khoshgoftar M, 2014. The effect of salinity on water status, proline, total soluble sugars and antioxidant activity of *Panicum antidotale* Retz. Journal of Science and Technology of Greenhouse 5 (18): 35-11.

Ferreira MI and Lourens AF, 2002. The efficacy of liquid seaweed extract on the yield of canola plants. South African Journal of Plant and Soil 19 (3): 159-161.

Fukutoku Y and Yamada Y, 1981. Diurnal changes in water potential and free amino acid contents of water-stressed and non-stressed soybean plants. Soil Science and Plant Nutrition 27 (2): 195-204.

Geholt HS, Purohit A and Shekhawat NS, 2005. Metabolic changes and protein patterns associated with adaptation to salinity in *Sesamum indicum* cultivars. Journal of Cell and Molecular Biology 4: 31-39.

Ghobanali M, Nojavan M, Heidari R and Farbodnia T, 2001. Soluble sugars, starch and proteins changes due to drought stress in Iranian chickpea (*Cicer arietinum* L.). Quarterly Journal of Science (Kharazmi University) 1: 38-53 (In Persian with English abstract).

Grattan SR and Grieve CM, 1992. Mineral element acquisition and growth response of plants grown in saline environments. Agriculture, Ecosystems and Environment 38 (4): 275-300.

Hanson AD, May AM, Grumet R, Bode J, Jamieson GC and Rhodes D, 2007. Betaine synthesis in chenopods: localization in chloroplasts. Proceedings of the National Academy of Science USA 82: 3678-368.

Heath RL and Packer L, 1968. Photoperoxidation in isolated chloroplast, I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125 (1): 189-198.

Hoagland DR and Arnon DI, 1950. The water-culture method for growing plants without soil. Circular of California Agricultural Experiment Station. 347. Second edition.

Irigoyen JJ, Einerich DW and Sánchez-Díaz M, 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiologia Plantarum 84 (1): 55-60.

Jiang Y and Hung B, 2001. Drought and heat stress injury to two cool-season turf grasses in relation to antioxidant metabolism lipid peroxidaion. Crop Science 41: 436-442.

Kameli A and Losel DM, 1996. Growth and sugar accumulation in durum wheat plants under water stress. New Phytology 132: 57-62.

Kao CH, 1981. Senescence of rice leaves. VI. Comparative study of the metabolic changes of senescing turgid and water–stressed excised leaves. Plant and Cell Physiology 22: 683–685.

Kaya C, Higges D and Kirnak H, 2001. The effects of high salinity (NaCl) and supplementary phosphorus and potassium on physiology and nutrition development of spinach. Journal of Plant Physiology 27 (3-4): 47-59.

Khan MA, Ungar IA and Showalters AM, 2000. The effect of salinity on the growth, water status and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.). Forssk. Journal of Arid Environment 45: 73-84.

Levitt J, 1980. Response of plants to environmental stresses. Vol 1. Chilling, Freezing and High temperature Stresses. Academic Press, New York.

Li G, Wan Sh, Zhou J, Yang Z and Qin P, 2010. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of Castor bean (*Ricinus communis* L.) seedlings to salt stress levels. Industrial Crops and Products 31: 13-19.

Lichtenthaler HK, 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350-382.

Lim JH, Park JK, Kim BK, Jeong JW and Kim HJ, 2012. Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. Food Chemistry 135: 1065-1070.

Masoumi A, Kafi M, Khazaei HR and Davari K, 2010. Effect of drought stress on water status, electrolyte leakage and enzymatic antioxidants of Kochia (*Kochia scoparia*) under saline conditions. Pakistan Journal of Botany 42 (5): 3517-3524.

Michel BE and Kaufmann MR 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology 51: 914-916.

Munns R, 2002. Comparative physiology of salt and water stress. Plant, Cell and Environment 25: 239-250.

Munns R and James RA, 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant and Soil 253: 201-218.

NasirKhan M, Siddiqui MH, Mohammad F, Masroor M, Khan A and Naeem M, 2007. Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. World Journal of Agriculture Science 3: 685-695.

Newton RJ, Bhaskaran S, Puryear J and Smith RH, 1986. Physiological changes in cultured sorghum cells in response to induced water-stress. II. Soluble carbohydrates and organic acids. Plant Physiology 81: 626-629.

Okcu G, Kaya MD and Atak M, 2005. Effect of salt and drought stress on germination and seedling growth of pea (*Pisum sativum*). Turkish Journal of Agriculture 29: 137-243.

Orcutt DM and Nilsen ET, 2000. The Physiology of Plants Under Stress: Soil and Biotic Factors. John Wiley and Sons, Inc., New York.

Paquine R and Lechasseur P, 1979. Observations sur one method dosage la libra dans les de planets. Canadian Journal of Botany 57: 1851-1854.

Pervize Z, Afzal M, Xi S, Xiaoe Y and Ancheng L, 2002. Physiological parameters of salt tolerance in wheat. Asian Journal of Plant Science 1: 78-481.

Prado FE, Boero C, Gallardo M and Gonzale JA, 2000. Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* wild seeds. Botanical Bulletin of Academia Sinica 41: 27–34.

Qasim M, Ashraf MM, Jamil AM, Rehman YSU and Rha ES, 2003. Water relations and gas exchange properties in some elite canola (*Brassica napus* L.) lines under salt stress. Annals of Applied Biology 142: 307-316.

Rahdari P, Tavakoli S and Hosseini SM, 2012. Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleracea* L.) leaves. Journal of Stress Physiology and Biochemistry 8 (1): 182-193.

Rezvani Moghaddam P, Karimpour H and Seyedi SM, 2015. Evaluation of yield and yield components of two wheat cultivars in different row cropping patterns. Iranian Journal of Field Crops Research 13(2): 232-238 (In Persian with English abstract).

Rhoads DM and McIntosh L, 1991. Isolation and characterization of a cDNA clone encoding an alternative oxides protein of S*auromatum guttatum* (Schott). Proceedings of the National Academy of Science of the USA 88: 2122-2126.

Sairam RK and Saxena DC, 2000. Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. Journal of Agronomy and Crop Science 184: 55-61.

Sannada Y, Ueda H, Kuribayashi K, Andoh T, Hayashi F, Tamai N and Wada K, 1995. Novel light-dark change of proline levels in halophyte (*Mesembryanthemum crystallinum* L.) and glycophytes (*Hordeum vulaare* L. and *Triticum aestivum* L.) leaves and roots under salt stress. Plant Cell Physiology 36 (6): 965-970.

Schobert B, 1977. Is there an osmotic regulatory mechanism in algae and higher plants? Journal of Theoretical Biology 68 (1): 17-26.

Schonfeld MA, Johnson RC, Carver BF and Mornhinweg DW 1988. Water relations in winter wheat as drought resistance indicators. Crop Science 28: 526-531.

Schutz M and Fangmeir E, 2001. Growth and yield responses of spring wheat (*Triticum aestivum* L. cv Minaret) to elevated CO2 and water limitation. Environmental Pollution 114: 187-194.

Taiz L and Zeiger E, 1991. Plant Physiology. The Benjamin/Cummings Publishing Company, Inc. 565 pages.

Weatherley PE, 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. New Physiology 49: 81-77.

Wise RR and Naylor AW, 1989. Chilling-enhanced photo-oxidation, the peoxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiology 83: 278-282.

Yu J, Sun L, Fan N, Yung Z and Huang B, 2015. Physiological factors involved in positive effects of elevated carbon dioxide concentration on Bermuda grass tolerance to salinity stress. Environmental and Experimental Botany 115: 20–27.

Zhao GQ, Ma BL and Ren CZ, 2007. Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Science 47: 123-131.